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REVIEW

Improving the translation of analgesic drugs to the clinic: animal models of neuropathic pain

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Neuropathic pain remains an area of considerable unmet clinical need. Research based on preclinical animal models has failed to deliver truly novel treatment options, questioning the predictive value of these models. This review addresses the shortcomings of rodent *in vivo* models commonly used in the field and highlights approaches which could increase their predictivity, including more clinically relevant assays, outcome measures and animal characteristics. The methodological quality of animal studies also needs to be improved. Low internal validity and incomplete reporting lead to a waste of valuable research resources and animal lives, and ultimately prevent an objective assessment of the true predictivity of *in vivo* models.

Abbreviations

MGS, mouse grimace scale; NME, new molecular entity; NNT, number needed to treat

Introduction

The International Association for the Study of Pain defines pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage' (Merksey and Bogduk, 1994). Pain is by definition a subjective multifaceted symptom which can only be 'measured' by self-report. Consequently, researchers and clinicians have to rely on subjective reports of presence, nature, location and intensity of pain in humans (Vierck *et al.*, 2008). Because of this, in animals, the presence or absence of pain cannot be directly measured and can only be inferred from the observation of surrogate behaviours. The present review concentrates on animal models used in analgesic development, we specifically focus on neuropathic pain but all the concepts discussed here are relevant to other type of pain. The prime focus of this review is on the use of animal models to predict clinical efficacy to the extent required for justification for initiation of a clinical development programme. However, it is important to note that use of animal models is also critical for processes involved earlier in the drug development pipeline, such as understanding of disease mechanisms and drug target identification. Neuropathic pain as defined by the International Association for the Study of Pain is 'pain caused by a lesion or disease of the somatosensory system' (Treede *et al.*, 2008; Jensen *et al.*, 2011). It can be associated with a plethora of diseases or lesions affecting the sensory nervous system and is associated with heterogeneous mechanisms and clinical presentations. It is also linked with a broad spectrum of other symptoms and clinical signs associated with both sensory loss and sensory gain (Baron *et al.*, 2010; Maier *et al.*, 2010).

Translation problem

Neuropathic pain has a prevalence of 7–8% in north-western European populations (Torrance *et al.*, 2006; Bouhassira *et al.*,

2008) and currently available treatment options for neuropathic pain are characterized by limited efficacy and a narrow therapeutic window (Dworkin *et al*., 2007; Finnerup *et al*., 2010). It is also difficult to predict treatment responses at the individual patient level (Dworkin *et al*., 2007; Finnerup *et al*., 2010). Pharmacotherapy only provides clinically meaningful pain relief in no more than 50% of patients suffering from neuropathic pain, with number needed to treat (NNT) across neuropathic pain conditions ranging from two to 12 depending on drug class (Finnerup *et al*., 2010). The neuropathic pain associated with certain conditions, for example human immunodeficiency virus (HIV)-associated sensory neuropathy, responds to very few interventions (Phillips *et al*., 2010). Additionally, pain relief is almost always partial rather than complete (around 30% greater than placebo) and associated with many side effects including cardiac toxicity, nausea, sedation and physical dependence (Borsook and Becerra, 2006; Dworkin *et al*., 2010a).

Even though the rate of Food and Drug Administration (FDA) approval of new molecular entities (NMEs) has been constant over the last 60 years (Munos, 2009), with an average of 23.5 NMEs approved during 2002–2011 (FDA, 2012), the number of NME applications has been declining steadily over the last 15 years, from 45 in 1996 to 23 in 2010 (FDA, 2011). This suggests that the number of drugs coming out of the Research and Development (R&D) pipeline is declining, despite an increase in pharmaceutical R&D expenditure over the same period (EFPIA, 2013). Additionally, an analysis of the success rate from first-in-man to registration during the period 1991–2000 across therapeutic areas shows that the level of attrition was very high, with a success rate of only 11%, meaning that only one in nine compounds were approved (Kola and Landis, 2004). There is nothing to suggest that attrition has improved over the last decade as a success rate of 6% was observed in 2009–2010 (Khanna, 2012). The breakdown analysis per therapeutic area provided by Kola and Landis (2004) showed that attrition was only slightly lower for arthritis and pain drugs, with a success rate of about 17% from first-in-man clinical trials to registration. In the past few years, several large- and medium-sized pharmaceutical companies have considerably downscaled their drug development activity in the pain field and little success has come out of the analgesic R&D pipeline in recent years. An analysis of analgesic drugs developed over the last 20 years shows that the majority of new drug launches result either from the design of novel formulations or dosing forms to improve the efficacy or safety of existing medicines, from compounds directed against known mechanisms, or the combination of existing drugs which improve efficacy or reduce side effects (Burgess and Williams, 2010). Out of the 59 drugs developed since 1960 and still in clinical use for the treatment of pain, only two-thirds were specifically developed as analgesics and the vast majority of these compounds developed as analgesics were directed against mechanisms already known to be involved in pain (Kissin, 2010). Most of the drugs associated with some degree of efficacy in the treatment of neuropathic pain, such as opioids, anticonvulsants or tricyclic antidepressants were originally introduced for other therapeutic indications, rather than being rationally developed using the classic animal-based drug development process. Pregabalin is sometimes painted as an exemplar translational success story for the use of animal models in the neuropathic pain field. However, when the evidence of clinical efficacy of pregabalin is objectively scrutinized, the conclusion is one of rather modest efficacy. We have examined this evidence objectively in a recent meta-analysis (Wiffen *et al*., 2013). Firstly, there is no top tier clinical trial evidence that pregabalin is efficacious for the treatment of neuropathic pain. For second tier evidence, pregabalin 600 mg does have a degree of efficacy in some selected neuropathic pain conditions {painful diabetic neuropathy [NNT for 50% pain relief = 6.3 (95% CI 4.6 to 10)], postherpetic neuralgia [5.6 (3.5 to 14)]} and central neuropathic pain [4.0 (3.1 to 5.5)]. However, reflecting on these data, even for these conditions only a minority of people treated achieve acceptably good pain relief (50%) with pregabalin. Furthermore, there are certain neuropathic pain conditions, such as HIV-related neuropathy where pregabalin has been shown to be ineffective (Phillips *et al*., 2010). Hardly a shining beacon of clinical effectiveness, although, of course, pregabalin is a major marketing success. Animal models were employed in the preclinical development of pregabalin, but an area of potential bias is that the findings from these preclinical studies only started to appear (Field *et al*., 1997) at about the same time as the pivotal clinical trials of gabapentin (Backonja *et al*., 1998; Rowbotham *et al*., 1998; Rice and Maton, 2001).

The paucity of new analgesic drugs contrasts with the amount of preclinical research reported. A PubMed search using the MeSH terms 'analgesic' and 'rodent' identified over 35 000 publications, with an average of 1350 publications per year over the last two decades (Figure 1). This raises questions about the utility and predictivity of animal models of pain. Although this review has its focus on the use of animal models in preclinical drug development, it should be noted in passing that there is also scope for methodological improvement in the clinical development phase. The difficulties of conducting early stage clinical proof of concept studies in neuropathic pain are pertinent; because nerve damage is a necessary prerequisite of neuropathic pain, the human surrogate models useful in inflammatory pain drug development are more of a challenge in neuropathic pain phase 1 proof of concept. Essentially, these studies have to be conducted in highly profiled patients. Furthermore, the design, conduct and analysis of phase 2 and 3 trials in neuropathic pain is not perfect and are being carefully scrutinized as an element of translational failure (see http://www.acttion.org and http://www.immpact.org; for examples see Dworkin *et al*., 2008; 2010b; 2013). Notwithstanding this, one could also reflect that in the unlikely event of a very highly effective neuropathic pain drug emerging from preclinical development, then perhaps such 'fine tuning' of clinical trials would become redundant. Returning to the use of animal models, Kontinen and Meert (2003) attempted to quantify the predictive validity of several rat models of neuropathic pain in a systematic review. The models reviewed included the chronic constriction injury, partial sciatic ligation, spinal nerve ligation and streptozocin-induced diabetes models in rats. Pharmacological data on behavioural outcomes in awake rats were compared with the clinical effectiveness of the drugs tested. Overall, it was found that the models were poorly predictive of clinical efficacy, with a specificity ranging from 0 to 60% depending on the model.

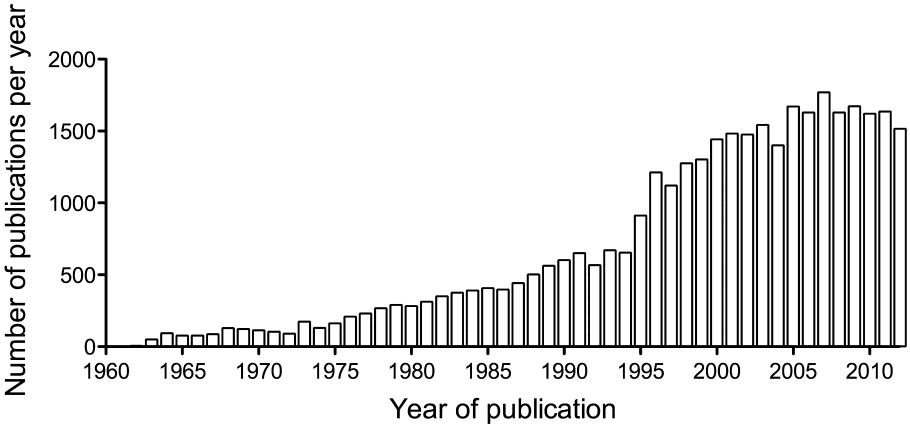

Figure 1

Number of articles published per year that reported experiments using rodents in the area of pain research. The graph was constructed based on a PubMed search using the MeSH terms: 'analgesic' and 'rodent'.

The low specificity numbers mean that animal studies detected an effect of drugs that had no efficacy in human clinical trials. These so called false positives lead to an unacceptable waste of resources, as ineffective drugs progress into further animal studies (e.g. toxicology, safety pharmacology) and clinical trials instead of being stopped at the efficacy stage. The sensitivity numbers were higher (61–88%), indicating that drugs, which displayed clinical efficacy usually also had efficacy in these animal models. The authors, however, recognized that their analysis had several limitations. First, the evidence used to determine the clinical efficacy of the drugs examined and used in the sensitivity calculations was not collected systematically and included weak evidence and clinical impression as well as evidence coming from systematic reviews. Drugs were considered effective if an effect had been observed in some kind of neuropathic pain; more rigid criteria might have decreased the sensitivity estimate for some of the models. Another issue was that of publication bias – very few animal studies reporting on ineffective drugs were identified in the systematic searches (Kontinen and Meert, 2003). Despite the limitations, this study provides a reminder that most commonly used animal models of neuropathic pain are not ideal and there is a real scope for improvement in the way pain research is currently carried out. An *in vivo* model is characterized by the characteristics of the animals used, the intervention or the disease resulting in neuropathic pain and the outcome(s) measured. Consideration should be given to the subject, the species and strain, as well as age and sex in relation to the clinical condition being modelled. Housing conditions can also influence the development of the condition and/or the outcomes measured. The assay – that is what is done to the animal to produce the condition of interest – can be either induced (surgically or via the injection of a chemical, a virus or immune mediators) or use animals presenting a naturally occurring disease. Chronicity is also an important part of the assay and consideration should be given to the duration of the disease and the timing of treatment and/or measurement of signs of pain. Again, the choice should be based on the human condition modelled. In terms of outcome measures, different tests can be used to infer signs of spontaneous pain, hypersensitivity and co-morbidities, which should provide a comprehensive picture of the pathophysiological condition developed by the animals and a complete assessment of the effect of putative analgesics with a spectrum of measures comparable with human clinical trials. Barriers to efficient clinical translation could arise either from issues with the models themselves – the subjects, the assays or the measures, and/or the way experiments are designed and reported. The present review explores potential refinements in each of these areas, which could improve the predictive validity of animal models of neuropathic pain.

Induction of neuropathic pain

In humans, neuropathic pain is a heterogeneous condition; patients present with various combinations of different signs and symptoms both within and between the different diseases and injuries associated with neuropathic pain (Baron *et al.*, 2010; Maier *et al.*, 2010). The incidence of sensory abnormalities (gain and loss) varies between, and within, pathophysiological conditions. For example, thermal hypersensitivity is observed in 39% of patients with peripheral nerve injury but only 8% of patients with polyneuropathy (Maier *et al.*, 2010). In contrast, the industry standards for the preclinical assessment of novel analgesics targeted at neuropathic pain are peripheral nerve trauma models, which rely on injury to a single nerve, usually the sciatic (Berge, 2011). The predominance of traumatic nerve injury models does not match the clinical situation, as shown by an analysis of the randomized clinical trials included in a systematic review (Finnerup *et al.*, 2005), which revealed that trials of patients with peripheral nerve injuries only represent 9% of the trials, while 53% and 21% of the trials were conducted in patients with peripheral polyneuropathies and post-herpetic neuralgia respectively (Rice *et al.*, 2009).

It would thus seem logical to select preclinical models that reflect more closely the precise pathophysiological condition studied in humans, rather than generalizing models as 'models of neuropathic pain'. A multitude of disease-specific models have been developed in recent years and, for example, include rodent models of peripheral nerve injury induced by anti-cancer (Authier *et al.*, 2000; 2003a,b; 2009; Polomano *et al.*, 2001; Flatters and Bennett, 2004; Ling *et al.*, 2007; Zheng *et al.*, 2011) or anti-retroviral therapy (Joseph *et al.*, 2004; Wallace *et al.*, 2007b; Huang *et al.*, 2013), diabetic neuropathy (Malcangio and Tomlinson, 1998; D'Almeida *et al.*, 1999), multiple sclerosis (Aicher *et al.*, 2004; Lisi *et al.*, 2012), lumbar radiculopathy (Olmarker *et al.*, 2002), central post-stroke pain (Wasserman and Koeberle, 2009), varicella zoster infection (Fleetwood-Walker *et al.*, 1999; Garry *et al.*, 2005; Hasnie *et al.*, 2007) or HIV-associated peripheral neuropathy (e.g. Wallace *et al.*, 2007a; Cao *et al.*, 2012). Although HIV neuropathy is difficult to replicate in rodents, as they are not a natural host for the virus, whereas the neuropathy can be induced in simian immunodeficiency virus infected non-human primates (Laast *et al.*, 2011).

There are, however, issues with induced models in laboratory species in that the incidence of pain (at least pain erroneously inferred from changes in reflex hypersensitivity – see Outcome measures section) is much higher than in humans, most of whom will *not* develop neuropathic pain after an injury or disease of the somatosensory system. However, an induced model which would develop neuropathic pain with an incidence as low as seen in humans would waste resources and require an unethically high number of animals per experiment as animals not developing the disease would not be used. Additionally, the underlying pain processes in induced models are uncertain in relation to naturally occurring pain, which questions their relevance to the human condition. Naturally occurring disease models can be used in pain research; they have stronger face and construct validity than the induced models and can be used to investigate the effect of putative analgesic compounds and study the central and peripheral neurobiology of pain in a natural disease state. For example, the effect of putative analgesics can be investigated in canine osteoarthritis, where the disease process is similar to humans (Clements *et al.*, 2006) or canine bone cancer (Brown *et al.*, 2009). Complex behaviours can be used as an indication of spontaneous pain in companion cats and dogs, via the use of an accelerometer placed in the animal's collar to measure the impact of pain on spontaneous activity in the home environment or pressure sensitive walkways to record body weight distribution on each limb. Additionally, questionnaires filled by the dogs' owners enable a measure of global quality of life, including the affective component of pain and how the pain interferes with typical activities (Lascelles *et al.*, 2010; Wernham *et al.*, 2011). Neuropathic pain can be investigated using such an approach – for example, diseases such as feline diabetic neuropathy (Mizisin *et al.*, 2002) might represent a more relevant model to study human diabetes than the streptozotocin rodent models, in which the resemblance to the human disease has been questioned (Tesch and Allen, 2007). Companion animals with spontaneous cancer could also be used in studies investigating cancer-related neuropathic pain or neuropathy induced by chemotherapy. Naturally occurring disease models do not circumvent the issue around outcome measures; there are no objective measures of pain. However, a salient feature is the comparability to human studies. Owners of companion animals are very familiar with their normal behaviour and approaches, so parental or caregiver questionnaires are used in the clinical assessment of non-verbal humans (e.g. children). There are disadvantages of using companion animals, which are mainly due to practicality and availability. Pet owners might not be willing to enrol their pets in a drug trial and with veterinary practices being broadly disseminated, it might be difficult to recruit these animals. These hurdles can, however, be overcome, as exemplified by the College of Veterinary Medicine at North Carolina State University which regularly advertises research programmes to recruit veterinary patients (http://www.cvm.ncsu.edu/docs/research.html). It would be unrealistic to expect naturally occurring disease studies to replace all rodent models but used in conjunction with some carefully chosen induced models in rodents in which the mode of induction and outcome measures are relevant to the clinical condition investigated, studies using companion animals could reduce the need for rodent studies and possibly help with the validation of some of the induced rodent models. Such studies can also be used to obtain more information and evaluate the efficacy of a putative analgesic before moving on to human clinical trials.

Reliance on existing analgesic drugs to investigate mechanisms and validate models can also be an issue. Animal models are often developed on the basis of mimicking clinical scenario and then optimized using known compounds which are sometimes incorrectly purported as 'gold standards', for example for a neuropathic pain model, gabapentin is often used as a comparative assay of analgesia. However, validating models with such compounds introduces a bias as known analgesic mechanisms will be preferred over novel mechanisms, which are not involved in the mode of action of gabapentin (Berge, 2011). Furthermore, gabapentin for that matter and other agents used clinically, such as tricyclic antidepressants or pregabalin, have limited effectiveness in the clinic – a recent meta-analysis estimated that the NNT with gabapentin or pregabalin for one patient to obtain 50% pain relief was over five across neuropathic pain conditions, and over 10 in patients with peripheral nerve injury (Finnerup *et al.*, 2010). Still, these drugs are used to unravel the mechanisms involved in rodent models of peripheral nerve injury (Andrews *et al.*, 2012; Morimoto *et al.*, 2012; Yoshizumi *et al.*, 2012) and such mechanisms might therefore not be clinically relevant. Another issue stemming from the lack of efficacious analgesics for neuropathic pain is the use of positive controls. Ideally, similar to the practice in clinical trials, when assessing the *in vivo* efficacy of a novel putative analgesic, a positive control group would be used. This is, however, difficult in neuropathic pain as no drug is unequivocally clinically effective enough to be used as such.

Outcome measures

Regardless of the species, there are no direct measures of pain. In humans, pain can be reported by the individual, but in animals, researchers rely on observing responses to presumed sources of pain. Historically, much emphasis has been placed on reflex withdrawal responses to sensory stimuli rather than measuring more complex, ethologically relevant behaviours from which the presence of spontaneous pain may perhaps be inferred. A review examining animal studies published in the journal Pain between 2000 and 2004 identified that the outcome most commonly assessed in preclinical studies is reflex hypersensitivity, with 90% of the papers exclusively reporting either thermal (48% of studies) or mechanical (42% of studies) hypersensitivity (Mogil and Crager, 2004). This finding is problematic for several reasons. First, there is a mismatch between animal studies and human trials. The predominant clinical feature of most neuropathic pain conditions is spontaneous (either continuous and/or paroxysmal) – as opposed to evoked – pain. While spontaneous pain is a symptom present in most neuropathic pain patients, sensory gain (as reflected in limb withdrawal measures) is much less frequent; mechanical hypersensitivity is seen in 57% of patients and thermal hypersensitivity in only 33% (Maier *et al*., 2010). Indeed, if paradoxical heat sensations were not included as a measure of sensory gain in such analyses, the prevalence of sensory gain would be much lower. Furthermore, while in humans these sensory gain features have some utility for sensory profiling, in animals they are incorrectly used as an efficacy parameter. In addition, there is limited correlation between thermal (hot and cold) and mechanical hypersensitivity and pain severity measured by questionnaires (Backonja and Stacey, 2004).

The majority of clinical trials include patients based on the level of pain intensity, rather than their sensory profile – although there is an increasing move to use sensory and symptom profiling as a stratification measure to predict analgesic responsiveness at the individual patient level (Attal *et al*., 2011; Freeman *et al*., 2014). In human trials, the primary efficacy measure is usually continuous spontaneous pain (see Table 1), which makes it difficult to draw any meaningful comparison with animal studies in which signs reflecting aspects of sensory gain are usually measured. It is likely that the difference in outcome measures partly contributes to the discrepancy between the findings of animal experiments and human clinical trials, as potential analgesics showing efficacy against hypersensitivity in animal models would have to be active under different neuropathological conditions to reduce spontaneous pain in patients (Lascelles and Flecknell, 2010).

Table 1

Comparison of the generic outcome domains generally measured in animal studies of neuropathic pain and corresponding clinical trials (reproduced with permission from Rice, 2010)

Outcome domain	Animal efficacy studies	Human randomized controlled trials
Evoked hypersensitivity	+	+/– (sensory profiling)
Spontaneous continuous pain	–	+[a]
Spontaneous paroxysmal pain	–	+/–
Co-morbidity	–	+
Physical function	–	+
Emotional function	–	+
Circadian rhythm disturbance	–	+
Adverse events	–	+
Global impression	–	+

[a]The usual primary efficacy measure.

Second, using reflex tests in animals presents an additional issue in that the mechanisms involved in the generation of reflexes do not include the cerebral cortex. Two conditions must be fulfilled to evaluate pain sensitivity: measures of pain should demonstrate transmission over nociceptive pathways to the cerebral cortex and require processing of sensory intensity in comparison with previous experiences (Vierck *et al*., 2008). Evidence from human fMRI studies shows that cortical regions are involved in both acute and chronic (including neuropathic) pain (Borsook and Becerra, 2006). Reflex tests such as paw withdrawal or tail flick may not involve cortical structures, but result in the activation of spinal and bulbospinal pathways (Lascelles and Flecknell, 2010). Behaviours, such as grooming of the injured paw or paw shaking following formalin injection and tail-flick upon thermal stimulation, have been observed in decerebrate animals (Matthies and Franklin, 1992). It is, however, possible to measure hypersensitivity using an operant testing paradigm rather than reflex measures. Operant responses, such as conditioned place preference, rely upon cerebral processing (Vierck *et al*., 2005) and yield findings that are more similar to the clinical impression than findings obtained using reflex testing (Vierck *et al*., 2008).

In recent years, novel measures have been investigated to infer signs of spontaneous pain in animals. Mogil *et al*. developed a method to assess pain using facial expression analysis. The mouse grimace scale (MGS) was adapted from a scale which is used in non-verbal human populations and consists in five facial features including three that are identical to those observed in humans – orbital tightening, nose bulge and cheek bulge – in addition to ear position and whisker change (Langford *et al*., 2010). The technique was useful to detect changes in a range of acute and inflammatory pain models, such as tail withdrawal from hot water or intraplantar capsaicin, but failed to detect changes in peripheral nerve injury models (chronic constriction injury and spared nerve injury). The analgesic effects of morphine and several NSAID analgesics could also be detected using the MGS as they reduced pain scores in mice challenged with cyclophosphamide-induced bladder cystitis (Langford *et al*., 2010) and in a postoperative pain assay (Matsumiya *et al*., 2012). Further work from the same laboratory showed that the MGS could be translated to the rat and enabled changes to be detected in inflammatory assays and following a laparotomy. These changes were also reversed by morphine (Sotocinal *et al*., 2011). Other groups have also looked at facial expression to assess pain in other species, for example rabbits (Keating *et al*., 2012).

Other analgesiometric tests have been developed based on the principle of looking at behaviours suppressed by pain – and therefore re-instated by analgesic drugs (Andrews *et al*., 2011). Andrews *et al*. used spontaneous burrowing as a behavioural endpoint, which is ethologically relevant to rodents. Burrowing activity (Deacon, 2006, 2009) in rats, measured as the amount of gravel displaced from a cylinder, was significantly reduced by three different techniques of peripheral nerve injury (tibial and L5 spinal nerve transections, and sciatic nerve ligation) and in an inflammatory model (intraplantar injection of complete Freund's adjuvant). This technique also detected the effect of analgesic drugs, and gabapentin and ibuprofen were shown to reverse the decrease in burrowing activity induced by peripheral nerve injury (up to 56 days post-injury) and complete Freund's adjuvant injection, respectively, at doses lower than those generally found effective in reflex response assays (Andrews *et al.*, 2012). Burrowing is also perturbed in a clinically relevant model of antiretroviral-induced sensory neuropathy, which is not complicated by the potential confound of motor deficits (Huang *et al.*, 2013). Burrowing has also been used as an indicator of postoperative pain in mice, measured as the amount of food pellets displaced; the decrease in burrowing activity following a laparotomy was reversed by the NSAID carprofen (Jirkof *et al.*, 2010). In this study, anaesthesia on its own reduced the percentage of animals displaying the burrowing behaviour for up to 12 h, demonstrating that a reduction in burrowing activity is not specific to pain. However, in contrast to the burrowing decrease induced by surgery, this was not reversed by carprofen (Jirkof *et al.*, 2010), indicating that pain is an important factor in burrowing reduction. The efficacy of analgesic drugs in this context does not, however, allow the distinction between whether this behaviour is an indicator of pain itself or the results of its co-morbidities, such as reduced well-being (Andrews *et al.*, 2012). The utility of burrowing as an assay across time has been demonstrated in the detection of the behavioural effects of hippocampal scrapie infection in mice. Repeated measurement of this behaviour across time started to detect abnormalities at about 14 weeks following infection, corresponding with the clinical course of the disease (Deacon *et al.*, 2005).

Other measures of spontaneous, voluntary activity, which have been investigated, include wheel running (Cobos *et al.*, 2012) and rearing (Matson *et al.*, 2007) in inflammatory models in mice and rats respectively. Some research groups have also used behavioural scores, recording behaviours such as arching of the back, pressing abdomen towards the floor or wobbling (Leach *et al.*, 2012), but these measures have predominantly been investigated in models of postoperative pain rather than neuropathic pain. Behaviours such as hypomotility, licking, lifting or shaking of the affected paw and gait changes have also been used to assess spontaneous pain in rat and mice models of nerve injury and diabetic neuropathy (e.g. D'Almeida *et al.*, 1999; Kontinen *et al.*, 1999; Benbouzid *et al.*, 2008). However, a recent, comprehensive study looking at over 20 strains of mice with two types of nerve injuries concluded that such behaviours could not reliably be used to measure spontaneous neuropathic pain (Mogil *et al.*, 2010b).

The activity of C-nociceptive fibres can also be recorded as an indication of spontaneous pain. Microneurography provides an objective measure with high translational value, as the technique can be used in both in peripheral neuropathic pain patients and rodent models of neuropathic pain models such as peripheral nerve injury and diabetic neuropathy (Serra, 2012). It represents a direct approach to assess the efficacy of putative treatments.

Neuropathic pain also has a wide impact on quality of life, mental health and ability to function and is associated with a number of co-morbidities in humans, such as sleep disturbance, social withdrawal, cognitive dysfunction, anxiety or depression (Mogil *et al.*, 2010a; Rice, 2010). In clinical trials, a broad range of measures and signs are usually measured as secondary outcomes and the IMMPACT group has provided recommendation on the domains that should be assessed (Dworkin *et al.*, 2008). Co-morbidities are used to describe the multifactorial nature of pain and measuring such behaviours in animal models would provide a complete picture of the animal's experience of chronic pain (Blackburn-Munro, 2004). Many studies have attempted to measure a range of complex pain-related behaviours in animal models of neuropathic pain, including increased thigmotaxis (predator avoidance) in an open arena (e.g. Hasnie *et al.*, 2007; Wallace *et al.*, 2007a,b,c; 2008; Hu *et al.*, 2009; Huang *et al.*, 2013), and elevated plus-maze behaviours (Roeska *et al.*, 2009a,b), sleep pattern disturbances (e.g. Andersen and Tufik, 2003) and various approaches to place preference (e.g. King *et al.*, 2009). Studying in animal experiments the signs which are affected by pain and contribute to a reduced quality of life is important to understand the precise action of putative analgesic drugs and improve the relevance of these studies to humans. This requires preclinical researchers to have a detailed understanding of the ethology of rodents and how such behaviours are influenced by pain, especially as a prey species (Barnett *et al.*, 2006; Barnett, 2009). What must be avoided is the temptation to anthropomorphize human emotions and behaviours to the rodent's world (Vasconcelos *et al.*, 2012).

However, measuring complex behaviours is challenging and the stability of such techniques easily perturbed by subtle changes related to environmental events and minor protocol variations. For example, predator avoidance behaviours in rats – and consequently, effect of drugs – are modulated by illumination levels and height of the platform in the elevated plus-maze test (Roeska *et al.*, 2009b). This variability can make consistent replication of these paradigms between laboratories and across time difficult. This is a particular challenge for preclinical drug development and a better understanding of these factors, and how to control them, is urgently required. Furthermore, there is considerable inter-animal variation in such behaviours, which can make group level effects difficult to interpret. More attention should be paid to within-subject changes in behaviours rather than group level averages – a straight parallel with the manner in which clinical trial analysis is moving away from group level effects to place more emphasis on the importance of individual patient responses (Moore *et al.*, 2013).

Animal characteristics

In the vast majority of preclinical pain experiments, the animals used are previously healthy, young, male and genetically similar rodents, which contrasts with the clinical populations who develop chronic neuropathic pain (Rice, 2010). In addition, neuropathic pain often develops in association with a disease, such as HIV, diabetes or cancer, rather than in healthy individuals. The profile of human patients affected by neuropathic pain also varies based on the aetiology, for example, in a recent study including over 1200 patients, women represented 64% of postherpetic neuralgia patients and 58% of these patients were over 69 years old; whereas peripheral nerve injury patients were predominantly males (55%) and younger, with 58% under 50 years old (Maier *et al*., 2010). The human population is also heterogeneous in terms of pain tolerance based on gender (Mogil, 2012) and ethnicity (Alabas *et al*., 2013). Additionally, responses to analgesic drugs differ between sexes, for example morphine is more potent in women but has a slower onset of action (Niesters *et al*., 2010). Interestingly, most rodent studies show greater opioid analgesia in males compared with females (see Dahan *et al*., 2008 for review). Further heterogeneity has been observed in laboratory animals, and strain (Mogil *et al*., 2005) and age (Pickering *et al*., 2006) have been shown to impact on the development of signs of pain. This highlights the importance of considering carefully the characteristics of animals used in preclinical studies, as conclusions will be dependent on these variables.

It is also reasonable to question the choice of species in experimental pain studies. Since the 1980s an overwhelming majority of pain experiments have used rats, with the use of mice steadily increasing (with the advent of transgenic technology) and almost matching rat usage over the last 15 years (Mogil, 2009). The use of prey species to investigate pain behaviours is arguable; displaying overt pain behaviour would not represent a survival advantage for rodents as it would make them more vulnerable to predators. It is conceivable that rodents may not display any overt signs of chronic spontaneous pain or at least interpretable behaviours would be geared to increases in predator-avoidance behaviours rather than displays of pain behaviours. Furthermore, rodents cannot model the complex neuroanatomy of the human brain, nor complex human emotions and behaviours associated with pain, and pathways known to be involved in the human experience and emotionality of pain are not present in rodent brains (Craig, 2009), this limits the utility of rodent models to study central pathways and stresses the importance of exercising caution when predicting human outcomes based on rodent data.

Alternatively, some experiments might not necessitate the use of mammalian organisms and could be carried out in lower order vertebrates such as zebrafish, which are economically advantageous and low maintenance. In teleost fish, both mechanothermal and mechanochemical nociceptors are physiologically similar to mammalian receptors and the pathways to the CNS are conserved (Sneddon, 2004). Fish can be used to study the basic fundamental mechanism of nociception or, in appropriate conditions, for the screening of analgesic drugs. Zebrafish are easily amenable to the production of transgenic lines because of their high breeding rate (100 offspring every 3 months), external fertilization and the feasibility of high-throughput phenotyping (Gonzalez-Nunez and Rodriguez, 2009). Additionally, the transparency of the embryo and the developing larvae facilitate injections to the nervous system and reporter gene expression can be followed *in vivo* (Sneddon, 2004). Behavioural studies can also be carried out to look at signs of spontaneous pain (Sneddon, 2009). Models of acute nociception and pain sensitization have also been developed in *Drosophila*. This organism can play an important role in deciphering the genetic and molecular mechanisms of pain (see Milinkeviciute *et al*., 2012 for review). Heat and mechanical nociception assays have been developed in *Drosophila* and can be used for high-throughput genetic and pharmacological screening *in vivo*. For example, the entire fly genome has been screened for heat nociception, resulting in a library of ‘pain genes’, two-thirds of which (~400 genes) conserved through to humans, including many that had never been linked to pain before (Neely *et al*., 2010). A model of nociceptive sensitization has also been described in *Drosophila*, where UV radiation results in thermal hypersensitivity. This model can be used to study signalling mechanisms and uncover novel potential therapeutic targets relevant to neuropathic pain. For instance, the importance of the Hedgehog signalling pathway in hypersensitivity identified in *Drosophila* was confirmed in rat models of inflammatory and neuropathic pain (Babcock *et al*., 2011).

Chronicity

In a recent trial of topical clonidine for the treatment of painful diabetic neuropathy, the average duration of diabetes was 10 years and the duration of pain, about 3 years (Campbell *et al*., 2012). In contrast, in animal studies, the effect of treatments is only tested a few weeks after diabetes induction (e.g. Schreiber *et al*., 2012; Sun *et al*., 2012). The same discrepancy is observed for pain of other aetiologies, for example post-herpetic neuralgia. While patients enter trials several years post-diagnosis (Rice and Maton, 2001), behavioural changes in rodent models of zoster-associated pain are only detected up to 10 weeks post-infection (Garry *et al*., 2005; Hasnie *et al*., 2007) and the effects of pharmacological treatments were investigated approximately 3 weeks post-infection (Hasnie *et al*., 2007). Thus, even considering the lifespan of laboratory rodents, which differ by a factor of about 35 (Rice *et al*., 2009), the duration of the disease tends to be shorter in animals compared with human patients. In preclinical models, signs of pain are measured during the initiation, or acute injury phase of the disease when inflammation may be a confounding factor and the neurobiology of pain – including mechanisms, co-morbidities and functional expression of drug targets – might differ from patients which would have had the painful condition for many years (Lascelles and Flecknell, 2010). Discrepancies in the duration of the measurement period post-treatment might also yield misleading results. For example, carbamazepine was found to be effective in a rat model of nerve injury 1 week post-surgery (Hahm *et al*., 2012), but in humans, it was shown to delay the onset of neuropathic pain following spinal cord injury, resulting in a lower incidence of neuropathic pain compared with the placebo-control group at the 1-month follow-up, but no difference were detected between the two groups after three months (Salinas *et al*., 2012). Preclinical and clinical studies also differ in terms of duration of treatment exposure. In preclinical models, drugs are usually given at high dose as a single administration, whereas in clinical settings they are titrated over several days for tolerability reasons (Berge, 2011). For example, in a clinical trial of patients suffering from post-herpetic neuralgia, gabapentin doses were increased over 2 weeks before reaching the test doses (Rice and Maton, 2001). This can lead to differences in plasma concentration and drug exposure. Whiteside *et al*. (2008) investigated five FDA-approved drugs for the treatment of neuropathic pain and compared drug exposure in humans with exposure in the rat spinal nerve ligation model. The minimal efficacious exposure in the rat model was between 1.2-fold (gabapentin) and 15-fold (duloxetine) greater than that measured in humans. These differences might reflect differences in drug distribution, especially across the blood–brain barrier, and differences in the antinociception mechanisms involved (Berge, 2011).

Design and reporting

The methodological quality and transparent reporting of animal studies is also a factor to consider when assessing the translational value of animal models (Rice *et al*., 2013). There is considerable scope for improving the standards to which animal research is conducted, especially with regard to means of addressing internal validity and experimental biases such as concealed allocation, randomization, observer blinding, reporting of withdrawals, declaration of conflicts of interest and details of sample size calculation (Kilkenny *et al*., 2009; Macleod *et al*., 2009). The field of pain research is not exempt from these issues (Rice *et al*., 2013). In a review of animal studies published in the journal *Pain* over a 6-month period in 2007, less than a third of studies were described as blinded or randomized (Rice *et al*., 2009) and only 15% reported both (Quessy, 2009). None of the 14 papers identified reported a power calculation to justify group sizes (Rice *et al*., 2009). In a more recent review looking at animal models of bone cancer pain, only 11% of the 150 publications identified reported random allocation to groups, a third mentioned blinded assessment of outcome and none reported sample size calculations (Currie *et al*., 2013).

Many studies have demonstrated the impact of bias on findings. Animal studies that do not report randomization and blinding are more likely to obtain a treatment effect (Bebarta *et al*., 2003). Systematic reviews in various domains of neuroscience, such as stroke, multiple sclerosis or Parkinson's disease, have demonstrated that studies which fail to report random allocation to experimental groups, blinded conduct of experiment or blinded assessment of outcome overestimate treatment efficacy and report an exaggerated effect size (Macleod *et al*., 2008; Vesterinen *et al*., 2010; Rooke *et al*., 2011). Failing to deal with subjective bias compromises the internal validity of an experiment and findings obtained are not robust and reproducible. Such studies are therefore wasting animals and other valuable resources. One could argue that these findings reflect incomplete reporting rather that poor methodological quality. However, if subjective bias was addressed in studies which failed to report it, we would not expect estimates of efficacy to be affected. Eisenach and Lindner (2004) also describe how findings in the rat spinal nerve ligation model of neuropathic pain have been influenced by the perceived clinical situation, with regard to the effects of intrathecal opioids and the role of the sympathetic system. The fact that rigorously blinded experiments were not able to replicate initial non-blinded studies indicates that the result of neuropathic pain studies can be affected by the expectations of the experimenter. In addition, studies are often not adequately powered, sample size calculation is virtually never reported in animal studies, including in the pain field (Rice *et al*., 2009; 2013; Currie *et al*., 2013). In the stroke field, it was estimated that preclinical studies only have a one in three chance of detecting a 20% difference in outcome (Macleod *et al*., 2009), which implies that two studies out of three are a waste of time, resources and animals. Although the face validity of animal models used in neuropathic pain research has improved over the recent years (see Induction section), the face validity of outcome measures remains a major hurdle (see Outcome measures section), in contrast with the stroke field in which a few objective outcome measures can be used and compared across species (e.g. infarct size). We would therefore argue that this estimation is conservative and is likely to be higher in neuropathic pain research.

Systematic review and meta-analysis are the method of choice to combine the results of multiple high-quality randomized controlled trials and to estimate the overall effectiveness of clinical interventions. The advantages of such an approach to ascertain efficiency of current models in predicting the clinical efficacy of putative analgesic drugs are obvious. In their review, Kontinen and Meert (2003) did not manage to assess the specificity of the models they investigated reliably, partly because the review only identified a limited number of studies reporting that the drugs tested were ineffective. This phenomenon is known as publication bias, when studies are less likely to be published if the data generated do not support rejection of the null hypothesis (i.e. for intervention studies, no difference between the treatment groups), or when an analysis or an outcome is selectively reported based on the direction of the effect. It has been widely recognized as a problem in clinical research, as it leads to incorrectly high expectations of efficacy and there have been calls to address this issue in the clinical pain field (Rowbotham, 2009). In animal research, a study investigating publication bias in the field of stroke estimated that in addition to the 1359 experiments identified, a further 214 experiments (16%) yielding negative results had been carried out but not reported; publication bias accounted for around one-third of the efficacy reported in systematic reviews (Sena *et al*., 2010). Additionally, an analysis of animal studies of neurological diseases, including over 4000 datasets, showed that there are too many animal studies with statistically significant results, which suggests strong selective analysis and outcome reporting biases (Tsilidis *et al*., 2013). There is no reason to believe preclinical pain studies are different in that respect and the true efficacy of putative analgesics in animal models cannot be reliably assessed if only some of the data generated by research teams around the world are made available.

There are also issues with the information included in studies that are published. Many have raised concerns about the underreporting of crucial aspects of the design and conduct of animal studies in general (Kilkenny *et al*., 2009; Muhlhausler *et al*., 2013) and in the field of pain specifically (Rice *et al*., 2009; 2013; Mogil *et al*., 2010a). The ARRIVE guidelines (Kilkenny *et al*., 2010a,b) were developed to address this issue and ensure that manuscripts contain the minimum information necessary to reproduce the experiments described or to assess their methodological quality, an essential step in meta-analysis. They have now been adopted by over 300 journals and major funding bodies, universities and learned societies (http://www.nc3rs.org.uk/ARRIVE). Implementation of the ARRIVE guidelines is essential to increase methodological transparency, which will in turn facilitate systematic reviews and meta-analyses of the animal literature and ensure that high-quality studies are recognized as robust, regardless of the direction of the outcome. Additionally, animal pain studies have been shown to be influenced by a plethora of experimental conditions, ranging from the animals' characteristics (Mogil, 2009) and housing conditions (Langford *et al.*, 2006) to room temperature (Hole and Tjolsen, 1993) and bedding texture (Robinson *et al.*, 2004). This information needs to be included in publications to inform interpretation of the findings and ensure that they can be replicated.

Research priorities

Neuropathic pain and pain in general are complex, multifactorial conditions which remain poorly understood and an unmet clinical need. In the last few decades, research has predominantly relied on *in vivo* animal models and will realistically continue to do so in the foreseeable future, but so far, despite a crucial need for more effective and safer analgesic drugs, little has translated to the clinic. In this review, we have discussed many ways by which the translational value of *in vivo* models could be improved. Tailoring models to the human condition investigated is essential. Recent efforts have focused on developing more clinically and ethologically relevant assays and outcome measures. Careful consideration should be given to the characteristics of the animal used and the design of the study to ensure that it matches the clinical situation. This will improve face and construct validity, but predictive validity should be assessed carefully. However, the environmental factors which govern variability in such measures, especially those that account for inter-laboratory variability, and how best to control these require elucidation. A systematic review is underway to assess objectively animal models of neuropathic pain and tease out the variables that improve their predictive value and reduce the burden on experimental animals (http://www.nc3rs.org.uk/researchportfolio-Sena). Issues related to subjective bias and selective or incomplete reporting also need to be addressed urgently

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Conflict of interest

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